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Fig. 2: Full length cDNA sequence **(SEQ ID NO: 1)** of a plant GnTI from potato (*Solanum tuberosum* L.) and amino acid sequence deduced therefrom **(SEQ ID NO: 2)**. By way of example, the complete cDNA of the membrane anchor containing *GntI* isoform from potato leaf tissue (A1) is illustrated. The EcoRI/NotI linkers at the 5' and 3' ends of the cDNA are highlighted by bold letters, the binding sites of the degenerate oligonucleotides used for obtaining the RT-PCR probe are underlined. In contrast to already published animal GnTI sequences, the protein sequence derived from the potato cDNA clones contains a potential N-glycosylation site: Asn-X (without Pro)-Ser/Thr, which is indicated by an asterisk. The region of the membrane anchor is highlighted in italics (aa 10 to 29). The start of the isoform (A8), which is potentially located in the cytosol, is indicated by an arrow.

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B, Comparison of the derived amino acid sequences of different plant *GntI*-cDNA clones. A_Stb-A1 **(SEQ ID NO: 2)**, GnTI from potato leaf; B_Ntb-A9 **(SEQ ID NO: 4)**, GnTI from tobacco leaf (A9); C_Atb-Full **(SEQ ID NO: 6)**, GnTI from *Arabidopsis thaliana*. Identical aa are highlighted in black, similar aa in light grey.

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Total RNA was isolated from potato and tobacco leaf tissue, and cDNA fragment of about 90 bp were amplified by means of RT-PCR in combination with degenerate primers (procedure analogous to ref. 31), which were derived from conserved amino acid regions of known GnTI sequences from animal organisms (sense primer 1* **(SEQ ID NO: 7)**, 5'-TG(CT) G(CT)I (AT) (GC) I GCI TGG (AC)A(CT) GA(CT) AA(CT) -3'; antisense primer 3* **(SEQ ID NO: 8)**, 5' -CCA ICC IT(AG) ICC (ACGT)G(CG) (AG)AA (AG)AA (AG)TC-3'; 30 pmol of each primer per 50 μl PCR assay at an annealing temperature of 55°C and 45 cycles). Following gel elution, the ends of the PCR products were repaired (i.e. blunt ended using DNA polymerase I and phosphorylated using T4 polynucleotide kinase) and clones into the EcoRV restriction site of pBSK (Stratagene). By comparison with known GnTI sequences between the primers (arrows), the identity of the derived amino acid sequences from the potato and tobacco RT-PCR products could be confirmed as being homologous; ⇒Q(R/M)QFVQDP(D/Y)ALYRS **(SEQ ID NO: 9)**⇐ (homologous aa are underlined). Of one clone each, radiolabelled probes were synthesized by means of PCR (standard PCR assay using degenerate primers as above, nucleotide mixture without dCTP, but instead with 50 μCi α-^{32}P-dCTP [>3000 Ci/mMol]), and different cDNA libraries were screened for *GntI* containing clones using the corresponding homologous potato or tobacco probes, respectively (procedure analogous to ref. 31; the stringent hybridization conditions have already been described in the text above). The cDNA libraries were prepared from mRNA of young and still growing plant parts (sink tissues). Following cDNA synthesis and ligating EcoRI/NotI adaptors (cDNAsynthesis kit, Pharmacia) EcoRI compatible lambda arms were ligated, those packaged and used to transfect *E. coli* XL1 Blue cells (Lambda ZAPII cloning and packaging system, Stratagene). Following amplification of the libraries, one full-length *GntI* clone each was isolated from a potato leaf sink library (A1 according to Fig. 2 and SEQ ID NO: 1) and a tobacco leaf sink library (A9 according to SEQ ID NO: 3), as well as two additional clones

from a tuber sink library (A6, A8). The deduced GnTI amino-acid sequences contain a potential N-glycosylation site, Asn-X (without Pro)-Ser/Thr, in contrast to those of animals. One of the tuber *GntI* cDNA sequences carries stop condons in all three reading frames in front of the first methionine (A8). The coding region shows high homology to the longer tuber clone (A6) (only 2 aa substitutions), but displays a completely different 5' non-translated region. Furthermore, the membrane anchor characteristic for the Golgi enzyme is missing, so that this GnTI isoform might be located in the cytosol. Sequence comparisons carried out by means of the gap or pileup option, respectively, and the box option of the gcg software package (J. Devereux, P. Haeberli, O. Smithies (1984) Nucl. Acids Res. 12: 387-395) indicate, that the deduced plant GnTI amino-acid sequences exhibit only 30-40% identity and 57-59% similarity to those of animal organisms (Fie. 3A), while they are highly homologous among each other (75-90% identity, Fig. 3B).

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The procedure in the case of *Arabidopsis thaliana* was analogous, wherein for the preparation of a specific probe first a partial *GntI* sequence was amplified by RT-PCR using *GntI* sense primer 4A **(SEQ ID NO: 10)**; 5'-ATCGGAAAGCTTGGATCC CCA GTG GC(AG) GCT GTA GTT GTT ATG GCT TGC -3'; HindIII restriction site underlined, BamHI printed in bold) and antisense primer 3*, as defined above. First, a 5'-incomplete cDNA clone was isolated from a phage library (Lambda Uni-Zap) using this probe. By means of a vector insert PCR, the missing 5'-terminus was amplified from another library (via [an] **a** unique SpeI restriction site in the 5' region) and assembled to yield a full-length cDNA sequence. The nucleic acid sequence determined by means of sequencing is listed in SEQ ID NO: 5.

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Into the SalI restriction site of the polylinker region (corresponding to the one of pUC18) of plant expression vector pA35 (ref. 29), a NotI linker was introduced subsequently to the fill-in of the ends (=pA35N), and the complete A1-*GntI*-cDNA (nucleotides 9 to 1657, according to the cDNA in Fig. 2) was inserted into pA35N *via* NotI (sense construct pA35N-A1s and antisense construct pA35N-Alas, respectively). The expression cassettes of the sense and antisense constructs, respectively, were isolated via the terminal restriction sites (filled-in NcoI restriction site, partial post digestion with HindIII) as a fragment of about 2410 bp and inserted into the EcoRI (filled-in) and HindIII restriction sites of the binary vector pBin19 (Ref. 30) (=pBin-35-Als and pBin-35-Alas, respectively). The EcoRI restriction site of the vector is restored by fusion with the equally filled-in NcoI restriction site of the fragment. By means of a standard PCR assay (sense primer **(SEQ ID NO: 11)**: KS sequencing primer (Stratagene) extended for PCR, 5'-GGC CCC CCC TCG AGG TCG ACG GTA TCG-3'; antisense primer **(SEQ ID NO: 12)**: 5'-GGGCCTCTAGA*CTCGAG* AGC (CT)AC TAC TCT TCC TTG CTG CTG GCT AAT CTT G-3', XbaI restriction site underlined, XhoI restriction site in italics), there was additionally amplified a 5'-fragment of the *GntI* cDNA at an annealing temperature of 50°C (nucleotides 9 to 261, according to the cDNA in Fig. 2 and SEQ ID NO: 1). The PCR product was digested with XbaI (within the antisense primer) and NotI (within the 5'-linker of the cDNA), isolated as a fragment of about 260 bp and cloned into pA35N (=pA35N-Al-short). The expression cassette of the short antisense construct was also inserted into pBin19 (=pBin-35-A1-short) as a EcoRI/HindIII fragment (about 1020 bp).

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Recombinant GnTI carrying 10 additional N-terminal histidine residues (His-tag) was produced in *E. coli* by means of the pET system (Novagen) and purified by metal-chelate affinity chromatography. A cDNA fragment comprising nucleotides 275-1395 of the potato *GntI* cDNA (corresp. to aa 75-446, Fig. 2 and SEQ ID NO: 1 and 2, respectively) was amplified by standard PCR (annealing temperature of 50°C, 30 cycles , ref. 31) (sense primer *GntI*-5' fus **(SEQ ID NO: 13)**: 5'-CATGGATCC CTC GAG AAG CGT CAG GAC CAG GAG TGC CGG C-3'; antisense primer *GntI*-3' stop **(SEQ ID NO: 14)**: 5'-ATCCCGGGATCCG *CTA* CGT ATC TTC AAC TCC AAG TTG-3'; XhoI and BamHI restriction sites, respectively, are underlined, stop codon initalics), and inserted into vector pET16b (Novagen) (=pET-His-Al) *via* the restriction sites of the synthetic primer (5'-XhoI-*GntI*-BamHI-3'). Following propagation and analysis in *E. coli* XL1-Blue (Stratagene) the construct was stored as a glycerol culture. Competent *E. coli* BL21 (DE3) pLysS cells (Novagen) were transformed with pET-His-Al for overexpression. Addition of IPTG (Isopropyl-1-thio-β-D-galactopyranoside, at 0.5-2 mM) to a BL21 culture in logarithmic growth phase, initially induces the expression of T7 RNA polymerase (from the bacterial chromosome), and thus, also the expression of the recombinant fusion protein under control of the T7 promoter in pET vectors (Novagen). By means of metal-chelate chromatography using TALON matrix (Clontech), recombinant potato GnTI was purified from induced BL21:pET-His-Al cells under denaturating conditions *via* its His-tag (manufacturer's protocol, Novagen), and their preparation was verified with respect to homogeneity by means of SDS-PAGE.

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Marked-up Copy of Amendments to the Claims 2, 31, 35, 47 and 48

2. (amended) Method according to claim 31, characterized in that for transformation an antisense or sense construct with respect to one of the cDNAs encoding N-acetyl glycosaminyl transferase I isolated from Solanum tuberosum, Nicotiana tabacum or Arabidopsis thaliana is used.

31. (amended) A method for the production of glycoproteins displaying [minimal, uniform] $GlcNac_2Man_5$-residues, comprising

(a) cultivating a transgenic plant, parts of transgenic plants or transformed plant cells, [and isolating the desired glycoprotein from the material cultivated, characterized in that] wherein

(i) the transgenic plant, parts of transgenic plants or transformed plant cells, respectively, is/are transformed with an antisense construct or a sense construct [, comprising] ;

(ii) the antisense construct or sense construct comprises an antisense DNA or a sense DNA with respect to the DNA [sequence for a gene or a cDNA for plant N-acetyl glucosaminyl transferase I] of claim 35 or a part thereof, wherein transformation of said antisense construct or sense construct results in [for] elimination or reduction of the activity of said N-acetyl glucosaminyl transferase in said transgenic plant, parts of transgenic plants or transformed plant cells; and [, wherein]

(iii) the antisense or sense construct optionally contains additional regulatory sequences for the transcription of the respective antisense or sense DNA;

and

(b) isolating the desired glycoprotein from the cultivated transgenic plant, parts of transgenic plants or transformed plant cells.

35. (amended) An isolated DNA, comprising a DNA molecule encoding a sequence or the complementary thereof, which is selected from the group consisting of:

SEQ ID NOs:1, 3 and 5;

a DNA sequence encoding the amino acid sequence of SEQ ID Nos: 2, 4 or 6;

a DNA sequence sharing a nucleotide identity of at least 70% with SEQ ID NOs:1, 3 or 5;

a DNA sequence encoding an amino acid sequence which shares an amino acid sequence of at least 75% with SEQ ID NOs:2, 4 or 6;

a DNA sequence which hybridizes under stringent conditions to SEQ ID NOs:1, 3 or 5, or the complementary thereof; and

a DNA sequence which hybridizes under stringent conditions to a DNA sequence, or the complementary thereof, which encodes SEQ ID NOs:2, 4 or 6.

47. (amended) A transgenic plant, transgenic seed, transgenic reproduction material, part of a transgenic plant or transformed plant cell, obtainable by

(a) integration of one or more antisense or sense DNA of claim 35 under the control of a promoter effective in plants, into the genome of a plant, or [by]

(b) viral infection by means of a virus containing one or more antisense or sense DNA of claim 35, for an extrachromosomal propagation and transcription of the antisense construct(s) in the plant tissue infected.

48. (amended) A transgenic plant, transgenic seed, transgenic reproduction material, part of a transgenic plant or transformed plant cell, obtainable by

(a) integration of one or more antisense or sense DNA of claim 36 under the control of a promoter effective in plants, into the genome of a plant, or [by]

(b) viral infection by means of a virus containing one or more antisense or sense DNA of claim 36, for an extrachromosomal propagation and transcription of the antisense construct(s) in the plant tissue infected.